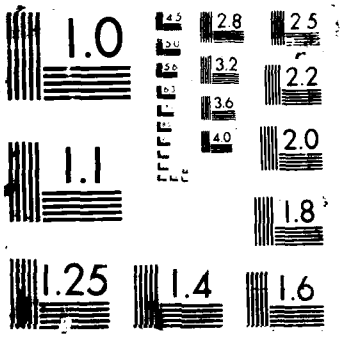
AD-A195 015 COCHLEAR HAIR CELL ELECTROCHEMISTRY: MECHANISMS FOR BIDIRECTIONAL TRANSDUCTION(U) JOHNS HOPKINS UNIV BALTIMORE MD SCHOOL OF MEDICINE W BROWNELL 30 JUN 88 1/1

UNCLASSIFIED N00014-87-K-0037 F/G 6/4 NL


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0

(U)

TIC FILE COPY

DTIC

(2)

REPORT DOCUMENTATION PAGE

AD-A195 815

L O 7 1988

D

NA	1b RESTRICTIVE MARKINGS: NA
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE: NA	3. DISTRIBUTION / AVAILABILITY OF REPORT: Distribution unlimited
4. PERFORMING ORGANIZATION REPORT NUMBER(S): Johns Hopkins University School of Medicine	5. MONITORING ORGANIZATION REPORT NUMBER(S): NA
6a. NAME OF PERFORMING ORGANIZATION: Johns Hopkins University; 6b. OFFICE SYMBOL (If applicable): NA	7a. NAME OF MONITORING ORGANIZATION: Office of Naval Research
6c. ADDRESS (City, State, and ZIP Code): Department of Otolaryngology-Head & Neck Surgery, Johns Hopkins University School of Medicine, 720 Rutland Ave., Baltimore, MD 21205	7b. ADDRESS (City, State, and ZIP Code): 800 N. Quincy Street, Arlington, VA 22217-5000
8a. NAME OF FUNDING / SPONSORING ORGANIZATION: Office of Naval Research; 8b. OFFICE SYMBOL (If applicable): ONR	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: N00014-87-K-0037 P 00001
8c. ADDRESS (City, State, and ZIP Code): 800 N. Quincy Street, Arlington, VA 22217-5000	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 61153N; PROJECT NO. RR04108; TASK NO. 441K704; WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)

(U) Cochlear Hair Cell Electrochemistry: Mechanisms for Bidirectional transduction

12. PERSONAL AUTHOR(S)
BROWNELL, William

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
	FROM 1/1/88 TO 6/30/88	1988 6 30	5

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	In vivo and in vitro studies of intracochlear electrical potential gradients. W.E. Brownell (Depts. of Otolaryngology-Head & Neck Surgery and Neuroscience, JHU, Baltimore, MD)
08			

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

A unique division of labor exists within the mammalian cochlea where electrochemical energy generated by one organ (the stria vascularis) is used by cells in another organ (the organ of Corti). Specifically, outer hair cells carry out bi-directional transduction (mechano-electrical and electro-mechanical) utilizing the energy of the endocochlear potential. We have measured intracochlear potential gradients in vivo to examine the fine structure of cochlear ionic currents both in silence and in response to acoustic stimulation [Brownell, W.E., Manis, P.B., & Zidanic, M., J. Acoust Soc. Am., 74:792-800 (1983); Brownell, W.E. Zidanic, M., & Spirou, G.A., Neurobiology of Hearing: The Cochlear, R.A. Altschuler, et, al. (Eds), Raven Press, 91-107 (1986).] The spatial profiles of potential gradient magnitude (as large as 20 mV/mm) and direction are compatible with a flow of cations (largely potassium) in a local circuit that is driven by an ionic pump in stria vascularis. The standing current (the "silent current") is large and can be measured in all three chambers of the mammalian inner ear. Mechano-electrical transduction by the hair cells in the organ

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ OTIC USERS	(U)
22a. NAME OF RESPONSIBLE INDIVIDUAL: Dr. Igor Vodyanoy	22b. TELEPHONE (Include Area Code): 202/696-4055; 22c. OFFICE SYMBOL: ONR

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

DISTRIBUTION STATEMENT A

Approved for public release;
Distribution Unlimited

R&T CODE: 4410001
DATE: 30-June-1988

PROGRESS REPORT ON CONTRACT N00014-87-K-0037

PRINCIPAL INVESTIGATOR: William E. Brownell, Ph.D.

CONTRACTOR: The Johns Hopkins University School of Medicine

CONTRACT TITLE: Cochlear Hair Cell Electrochemistry: Mechanisms for Bidirectional Transduction

START DATE: 1 January 1987

RESEARCH OBJECTIVE: To determine the cellular mechanism responsible for outer hair cell (OHC) electromotility. Our working hypothesis is that the electrically evoked movements of OHCs result from electo-osmotic movement of cytoplasm in the cell's laminated cisternal system. More specifically, we postulate that intracochlear potential gradients associated with acoustic transduction drive intracellular fluids through an electo-osmotic "pump" formed by the plasma membrane and the morphologically unique laminated cisternal membranes.

PROGRESS since 1-January-1988

1. MEASURING AXIAL POTENTIAL GRADIENTS IN OHC: Our working hypothesis requires that axial potential gradients exist in the outer hair cells. We are measuring the cable properties of the outer hair cell to determine if potential grandients of sufficient magnitude can be maintained by the cell. A command voltage is presented with the electrode amplifier in voltage clamp to a tight seal whole cell electrode (electrode 1) attached near either the synaptic cr stereociliar end of the cell. The current required to follow the command voltage is monitored and the series resistence of the electrode is calculated from the spectral impedance function or from hyperpolarizing step responses. A second whole cell electrode (electrode 2) is attached near the opposite end of the cell and the voltage measured at this location is compared to the series resistence corrected command voltage. Results from these experiments are presented is figures 1-3.

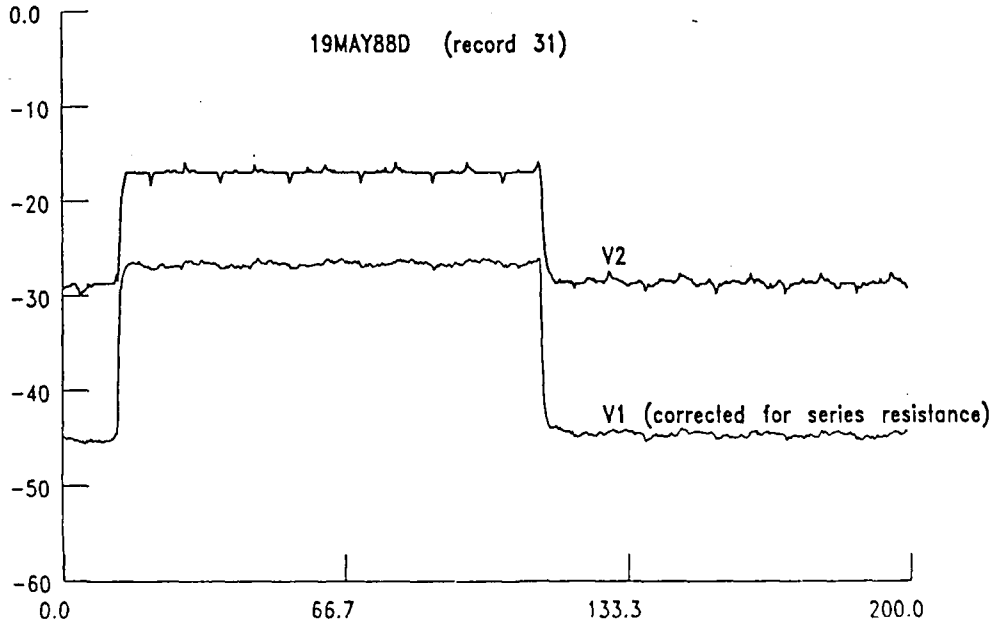


Figure 1

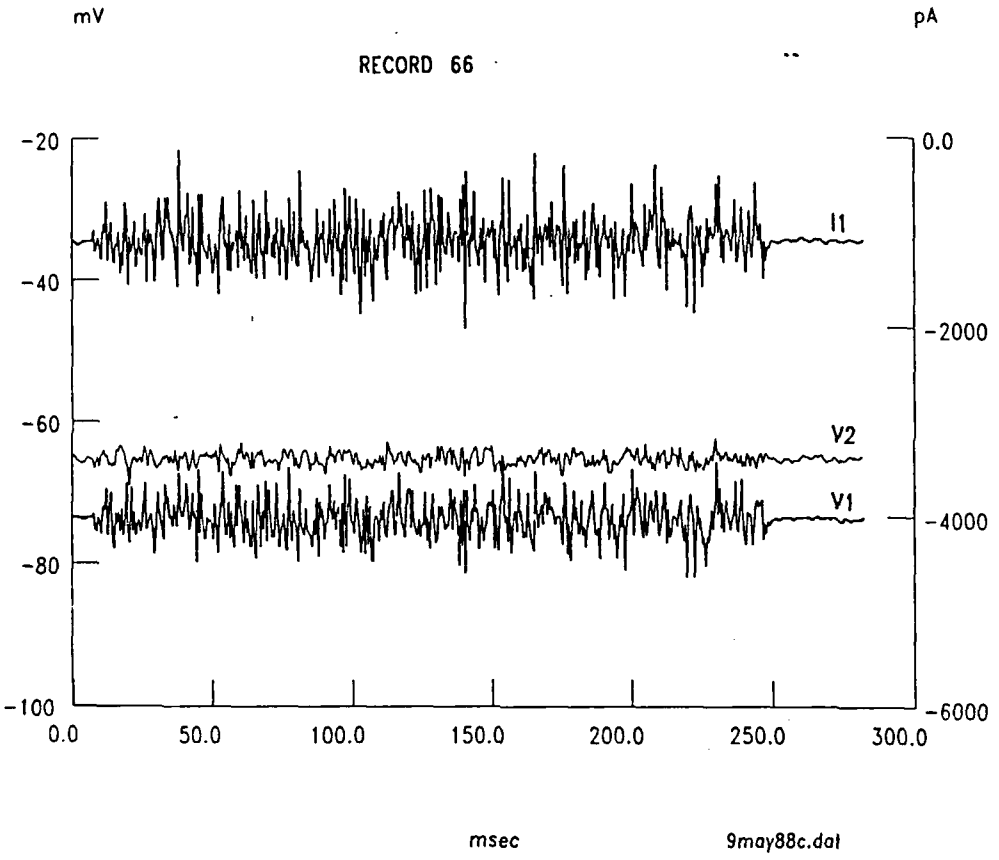


Figure 2

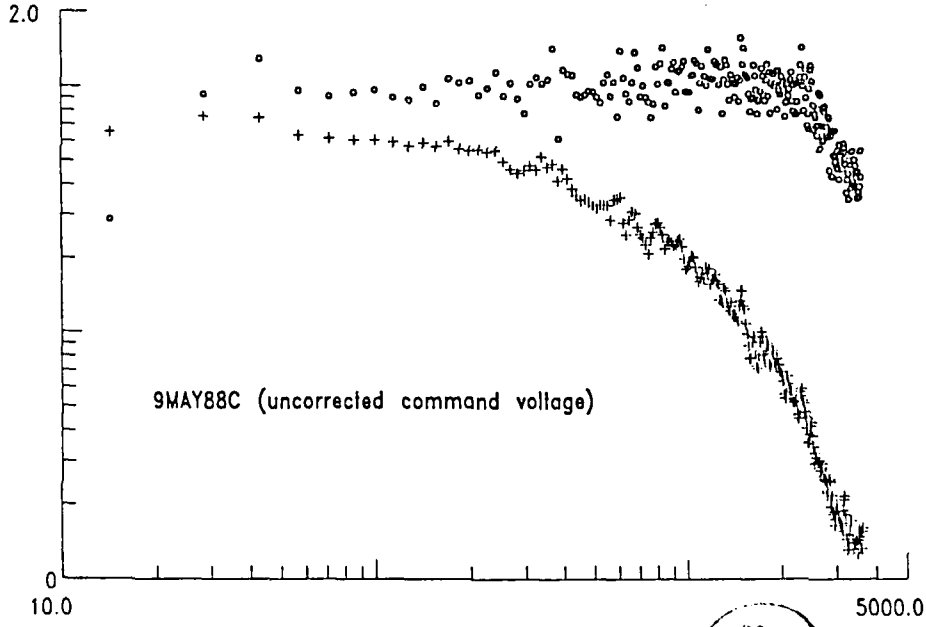


Figure 3

Figure 1 shows a voltage decrement over the 30 μm distance between electrode 1 (V1) and electrode 2 (V2).

Figure 2 shows a single presentation of a white noise command voltage in the bottom trace and the voltage measured 25 μm away is shown in the middle trace. The current required to produce the command voltage is presented in the top trace. Comparison of the voltage traces shows a voltage decrement that is greater for the higher freqency components of the signal.

The spectral composition of the command voltage (electrode 1) and the remotely recorded voltage (electrode 2) are shown in the top and bottom plots (respectively) in Figure 3. The voltage decrement and low pass filtering we observe is consistent with the existence of cable properties but we can not yet state unequivocally whether the behavior results from true passive cable properties or the possibility that the whole cell electrodes introduce significant error in the form of poor seals to the plasma membrane. Recordings made with our tightest seals have produced paradoxical negative voltage decrements.

2. POSSIBLE INVOLVEMENT OF POLYAMINES IN HAIR CELL TRANSDUCTION: The anti-neoplastic drug, difluoromethylornithine (DFMO), has caused reversible sensori-neural hearing loss in clinical tests on humans. DFMO is a specific blocker of the enzyme ornithine decarboxylase and blocks the production of polyamines. We have developed a guinea pig animal model that mimics the reversible hearing loss seen in humans. Over 25 animals have been processed since the last report. Morphological studies confirm the involvement of the laminated cisternal system in the hearing loss measured with brainstem evoked response. The highly polycationic nature of the polyamines suggest their possible involvement in electrokinetic phenomena.

3. FABRICATION OF NEW PHOTOMETRIC AMPLIFIER: We have designed and begun fabrication of a new photometric amplifier after finding dificiencies in our initial design. The image of electrically stimulated outer hair cells is projected out of the microscope onto a linear position detector. The detector's output is differentially amplified and either signal averaged or fed into a lock-in analyzer prior to signal averaging. This configuration permits the measurement of high frequency, low displacement movements of hair cells. We have used the old amplifier to make preliminary measures of the effect of temperature change on the movements (see work plan) and observed only small differences with temperature change. Software development has begun for computer control of the lock-in analyzer so that data collection may be automated.

4. OBSERVATION OF A NEW TYPE OF ELECTRICALLY INDUCED SHAPE CHANGE: We have become aware of a another type of electrically induced outer hair cell shape change over the last six months. Depolarization leads to the loss of fluid from the cell and hyperpolarization leads to an increase in cell volume. It requires several minutes to manifest itself and is most conspicuous when the holding potential is moved more than 50 mV to either side of the resting membrane potential. The previously described, high frequency movements, in contrast, appear to occur with little or no change in cell volume. High frequency movements eventually disappear as the cells lose their turgor during depolarization and reappear after several minutes of moving the holding potential to a hyperpolarizing value. The mechanism responsible for the slow, reversible, electrically induced shape change and its significance for cochlear transduction have yet to be established.

5. MORPHOLOGICAL EXAMINATION OF MEMBRANE SURFACE CHARGE: We have previously observed that the lectin FITC-HPA binds intracellularly in the outer hair cell suggesting that glycoconjugates with terminal N-acetyl-D-galactosamine residues exist inside the plasma membrane. If the intracellular binding of HPA indicates a strongly polyanionic surface

charge on the membranes of the intercisternal spaces, the resulting surface potential could increase the magnitude of the electrokinetic events postulated to be associated with OHC electro-motility. Our initial observation was based on the binding of HPA lectin purified by a French biological supply firm and we were unable to demonstrate the binding with the American firm SIGMA's HPA lectin. We have recently determined that this was due to the fixative used and now obtain identical result with the SIGMA product. SIGMA also has HPA bound with colloidal gold and the enzyme horseradish peroxidase which produce electron opaque products at the binding site. We should now be able to determine the intracellular locus of the glycoconjugate to which HPA is binding.

WORK PLAN:

1. MICROPHOTOMETRICALLY CHARACTERIZE THE DYNAMICS OF OHC CELL SHAPE CHANGES in response to step, pulse and sinusoidal electric stimulation. By measuring the movement magnitude and phase we will establish mechanical frequency response properties. These experiments will establish normative data with which to compare experimental results. Our newly implemented white noise analysis paradigm will permit the rapid collection of data. Potential gradient measures will be extended in order to provide as complete a discription of the gradient driving the hypothesized electrokinetic response. Our ability to place two whole cell electrodes on the cell permits the maintenance of controlled potential gradients within the cell. An alternative hypothesis for the motile mechanism is based on a postulated protein with piezoelectric like properties that is sensitive to the transmembrane potential. The null hypothesis for this possibility is that the movements occur under conditions in which there is no change in the net transmembrane potential. We can introduce voltage steps that are equal in magnitude but of opposite polarity at the two ends of the cell. If no movements occur it supports a transmembrane dependence, alternatively, their presence must be a function of the applied potential gradient in order to support our working hypothesis in which the movements are a function of an axial potential gradient.

2. MEASURE THE EFFECT ON OHC ELECTROMOTILITY OF MANIPULATIONS THAT CAN AFFECT ELECTRO-OSMOSIS: Manipulations include administration of substances capable of modifying the cell surface charge and parametrically varying the ionic composition of the bathing media. We will use aminoglycosides, polyamines and test hair cells taken from DMFO treated animals. The recently observed slow change in fluid volume that follows changes in the holding potential will also be used. The observed loss in rapid electromotility with a loss in cell turgor suggests that a motile mechanism requires a modest hydrostatic pressure in the cell.

Changes in temperature should produce a change in OHC dynamics. The equation describing the velocity of movement in electro-osmosis is:

$$\vec{v} = \frac{\vec{E}\epsilon\zeta}{4\pi\eta}$$

where $\vec{v}$ is the velocity of fluid flow
$\vec{E}$ is the potential gradient
ϵ is the dielectric constant of the medium
ζ is the zeta potential
η is the viscosity of the moving fluid.

The equation describes electro-osmotically driven fluid velocity as being inversely proportional to viscosity. For a liquid, viscosity is roughly related to temperature by:

$\eta = Ae^{\frac{\beta}{T}}$; so that electro-osmotic fluid velocity is: $\vec{v} \propto \left[Ae^{\frac{\beta}{T}}\right]^{-1}$

This relationship is postulated to describe the flow of cytoplasm between the membranes of the laminated cisternae in response to a potential gradient, which, in turn, may drive pressure changes within the cell, generating the conformational changes we detect as movement. While the above equation does not describe the velocity of the cell movement we will record, changes in electro-osmotic flow caused by temperature should elicit proportional changes in movement if other variables remain constant. Experimental data collected on the temperature dependence of muscle contraction have shown about a six fold change in contraction velocity over the same temperature range we will be using in our protocol. Expected electro-osmotic velocity changes should be about two fold over the same range. A measurable difference in the velocity of movement will prove a valuable test of our working hypothesis.

3. DETERMINE THE CELLULAR LOCALIZATION OF HPA LECTIN BINDING. HPA lectin, colloidally bound with gold or horseradish peroxidase will be used to determine where the HPA lectin binds within the cell using transmission electro-microscopic techniques. This work will be done in collaboration with Drs. Pablo Gil-Loyzaga (Madrid) and Peter Santi (Associate Professor, U. Minnesota).

INVENTIONS: None. No potentially patentable devices.

PUBLICATIONS AND REPORTS:

1. A manuscript authored by Gil-Loyzaga, P.E., and Brownell, W.F., entitled "Wheat germ agglutinin and Helix Pomatia lectin binding on cochlear hair cells" is in press in Hearing Research

2. An abstract authored by Jansen, C.J., Mattox, D.E., Miller, K.D., and Brownell, W.E., entitled "An animal model of hearing loss from alpha-difluromethylornithine (DFMO)," was published in the Abstracts of the Midwinter Research Meeting of the Association for Research in Otolaryngology 11 (1988) 257.

3. An abstract authored by Zidanic, M., and Brownell, W.E., entitled "Two-dimensional analysis of cochlear microphonics in the guinea pig cochlea," was published in the Abstracts of the Midwinter Research Meeting of the Association for Research in Otolaryngology 11 (1988) 169.

4. An abstract authored by Brownell, W.E., entitled "*In vivo* and *in vitro* studies of intracoclear electrical potential gradi[illegible]," will be published J. Acoust. Soc. Am. as part of a special symposium on Hair Cell Transduction and Cochlear Frequency Analysis.

5. An invited lecture entitled, "Outer hair cell response properties and the mechanism for electro-motility" was presented by W. Brownell as part of an international symposium entitled "Current Concepts of Hair Cell Function" held in Ann Arbor, Michigan between June 11-15, 1988.

TRAINING ACTIVITIES: An undergraduate, graduate, post-doctoral fellow and three Otolaryngology - Head & Neck Surgery residents have participated in portions of the research.

Women or minorities - 3 women
Non-citizens - 1 citizen of France

DISTRIBUTION LIST FOR REPORTS

ONR MEMBRANE ELECTROCHEMISTRY PROGRAM

Dr. Martin Blank
Department of Physiology
Columbia University College
of Physicians and Surgeons
630 W. 168th Street
New York, NY 10032

Dr. William E. Brownell
Department of Otolaryngology-HNS
Johns Hopkins University
School of Medicine
720 Rutland Avenue
Baltimore, MD 21205

Dr. Marco Colombini
Department of Zoology
University of Maryland
College Park, MD 20742

Dr. Michael A. Cusanovich
Department of Biochemistry
University of Arizona
Tuscon, AZ 85721

Dr. D. W. Deamer
Department of Zoology
University of California
Davis, CA 95616

Dr. Edward A. Dratz
Department of Chemistry
Montana State University
Bozeman, MT 59717

Dr. Harvey M. Fishman
Department of Physiology and
Biophysics
University of Texas Medical Branch
Galveston, TX 77550

Dr. Sol M. Gruner
Department of Physics
Jadwin Hall
Princeton University
P. O. Box 708
Princeton, NJ 08544

Dr. Felix T. Hong
Department of Physiology
Wayne State University
540 E. Canfield Avenue
Detroit, MI 48201

Dr. Huey W. Huang
Department of Physics
Rice University
Houston, TX 77251

Dr. Israel R. Miller
Department of Membrane Research
The Weizmann Institute of Science
Rehovot 76100
ISRAEL

Dr. V. Adrian Parsegian
Laboratory of Chemical Biology,
NIADDK
Room 9N-307
Building 10
Bethesda, MD 20892

Dr. Davis S. Perlin
Department of Biochemistry
Public Health Research Institute
455 First Avenue
New York, NY 10016

Dr. H. Gilbert Smith
EG & G Mason Research Institute
57 Union Street
Worcester, MA 01608

Dr. Michael E. Starzak
Department of Chemistry
State University of New York
Binghamton, NY 13901

Dr. H. Ti Tien
Department of Physiology
Membrane Biophysics Laboratory
Michigan State University
East Lansing, MI 48824

Dr. Tian Y. Tsong
Department of Biological Chemistry
Johns Hopkins University
School of Medicine
725 N. Wolfe Street
Baltimore, MD 21205

Dr. Peter Vanysek
Department of Chemistry
Northern Illinois University
De Kalb, IL 60115

ONR MEMBRANE ELECTROCHEMISTRY PROGRAM

Dr. Howard Wachtel
Dept. of Electrical & Computer Eng.
University of Colorado
Campus Box 425
Boulder, CO 80309

Dr. James C. Weaver
Div. Health Sciences & Technology
Room 20A-128
Massachusetts Institute of Tech.
Cambridge, MA 20742

Dr. George S. Wilson
Department of Chemistry
University of Kansas
Lawrence, KS 66045

Annual Final and Technical Reports

ADMINISTRATORS

Dr. Igor Vodyanoy, Code 1141SB (2 copies)
Scientific Officer, Biophysics
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Administrator (2 copies) (Enclose DTIC Form 50)
Defense Technical Information Center
Building 5, Cameron Station
Alexandria, VA 22314

Administrative Contracting Officer
ONR Resident Representative
(address varies - obtain from contract or your business office)

Dr. Robert J. Nowak, Code 1113ES
Scientific Officer, Electrochemical
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Program Manager
Biological/Human Factors Division
Code 125
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Program Manager Defense Technical
Support Technology Directorate
Office of Naval Technology, Code 223
800 N. Quincy Street
Arlington, VA 22217-5000

Annual and Final Reports Only (one copy each)

DoD ACTIVITIES

Commander
Chemical and Biological Sciences Division
Research Army Research Office, P. O. Box 1221
Research Triangle Park, NC 27709

Head
Biomolecular Engineering Branch
Code 6190
Naval Research Laboratory
Washington, DC 20375

Directorate of Life Sciences
Air Force Office of Scientific
Bolling Air Force Base Research
Washington, DC 20332

Final and Technical Reports Only — NO

Director, Naval Research Laboratory (6 copies)
Attn: Technical Information Division, Code 2627
Washington, DC 20375

END

DATE

FILMED

9-88

DTIC